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Evolutionary Change in Brain Size of Bats

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Abstract

It has been widely recognized that mammal brain size predominantly increases over evolutionary time. Safi et al. [2005] questioned the generality of this trend, arguing that brain size evolution among bats involved reduction in multiple lineages as well as enlargement in others. Our study explored the direction of change in the evolution of bat brain size by estimating brain volume in fossil bats, using synchrotron-radiation X-ray tomographic microscopy (SRXTM). Virtual endocasts were generated from 2 *Hipposideros* species: 3 specimens of Oligocene *H. schlosseri* (~35 Ma) and 3 of Miocene *H. bouziguensis* (~20 Ma). Upper molar tooth dimensions (M^2 length x width) collected for 43 extant insectivorous bat species were used to derive empirical formulae to estimate body mass in the fossil bats. Brain size was found to be relatively smaller in the fossil bats than in the average extant bat both with raw data and after allowing for phylogenetic inertia. Phylogenetic modeling of ancestral relative brain size with and without fossil bats confirmed a general trend towards evolutionary increase in this bat lineage.

Introduction

Jerison's classic 1973 text on evolution of the brain in vertebrates provided numerous examples clearly documenting a general increase in brain size over time in many mammal lineages. He confirmed an overall trend in the fossil record that had been progressively recognized since the time of Marsh [1874]. More recent studies of large data sets have clearly confirmed Jerison's interpretation for 3 orders of mammals: Primates [Martin, 1990; Martin and Isler, 2010], Cetacea [Marino et al., 2004] and

Carnivora [Finarelli and Flynn, 2007]. Moreover, the same trend has now been confirmed with respect to very early mammals [Rowe et al., 2005].

It was long thought that brain size is related to body surface area [Jerison, 1973], but it was subsequently proposed that brain size may instead be linked to metabolic turnover [Martin, 1981; Mink et al., 1981; Armstrong, 1983]. This alternative interpretation was proposed partly because brain tissue has a high energetic cost and partly because scaling of brain size parallels scaling in basal metabolic rate (BMR) rather than that of surface area. However, relative brain size shows a five-fold range of variation on either side of the average condition in mammals. Such wide variation greatly exceeds the relatively modest two-fold variation in adult BMR relative to the average condition. So brain size must be influenced by other factors in addition to BMR, and availability of resources during early development is a prime candidate. Recognition of this led to formulation of the Maternal Energy Hypothesis [Martin, 1996; Martin and Isler, 2010]. According to this hypothesis, a larger brain reflects an increase in maternal resources devoted to the developing offspring. Over evolutionary time, a general trend towards an increased level and efficiency of commitment of maternal resources could explain the empirical finding that brain sizes tend to increase in mammals. This general tendency would be promoted by pervasive benefits of increased brain size and specific responses to new behavioural requirements.

However, the long-accepted generalization that mammal brains have typically tended to increase in size over evolutionary time has recently been questioned. With specific reference to bats (order Chiroptera), Safi et al. [2005] proposed that brain size evolution involved reduction rather than enlargement in multiple lineages. The proposal

that a decrease in brain size was widespread during bat evolution was explicitly linked to the high energetic cost of maintaining a large brain. But the inference of widespread brain size reduction was exclusively based on a theoretical analysis of brain size in extant bats.

Safi et al. [2005] did not themselves allocate change in brain size to individual lineages, but they provided the requisite data to Niven [2005], who did so. According to Niven's figure 1, the prevalent trends were for brain size decrease in 7 families (Emballonuridae, Hipposideridae, Molossidae, Noctilionidae, Rhinolophidae, Rhinopomatidae and Vespertilionidae) and brain size increase in 5 families (Craseonycteridae, Megadermatidae, Mormoopidae, Nycteridae and Phyllostomidae).

Safi et al. [2005] went on to suggest that in fast-flying bats brain size decreased relative to the ancestral condition because reduced body mass was needed to forage in open spaces. According to this proposal, the primary requirement was for flight efficiency. The energetic cost of a large brain was conceivably disadvantageous, so sensory needs were relaxed. By contrast, it was argued that brain size might be expected to increase over time in bats that have large wings for manoeuvering in highly structured habitats. Safi et al. proposed that bats living in such dense habitats would require better spatial memory and hearing for navigation [see also Safi and Dechmann, 2005]. When effects of body mass and phylogeny were taken into account by using a phylogenetic least-squares approach (PGLS), wing size was found to be positively correlated with brain size in insectivorous bats.

Consideration of fossil specimens is needed to test the claim that reduction in brain size was a widespread occurrence during the evolution of bats, but this is no easy task. Jerison [1973] emphasized the great rarity of fossil bat specimens indicating brain

morphology or size, and there appears to be no published record of relative brain size in any species. The absence of published information on brain size in fossil bats is particularly due to their very small body sizes and to the associated rarity of specimens preserved in three dimensions. For instance, the well-known Eocene bats from Wyoming in the U.S.A. (*Icaronycteris*) and from Messel in Germany (*Archaeonycteris*, *Hassianycteris*, *Palaeochiropteryx*) are documented exclusively by flattened specimens that preclude any direct measurement of brain size. Because prior information on relative brain size in fossil bats was lacking in the literature, collection of new data to meet this need was required. It was necessary to find suitably preserved fossil bat specimens and to apply study methods appropriate to their very small size.

Materials and Methods

A two-pronged approach was adopted to obtain and interpret fossil evidence to assess direction of change in the evolution of relative brain size in bats: (1) Synchrotron-radiation X-ray tomographic microscopy (SRXTM) was used to generate virtual endocasts for determination of brain volume of 6 Oligocene/Miocene bat specimens. (2) Standard dental dimensions were collected for 43 extant microbat species and used to calculate an empirical formula permitting estimation of body size in fossil bats. Body mass values were taken from Jones et al. [2009].

Higher-level classification of bats (order Chiroptera) requires comment. Traditionally, Chiroptera were divided into two suborders: Microchiroptera (23 families of echolocating microbats) and Megachiroptera (megabats in the single family Pteropodidae). However, reports based on molecular evidence indicated that

microchiropterans are polyphyletic, with 5 families (Craseonycteridae, Hipposideridae, Megadermatidae, Rhinolophidae, Rhinopomatidae) being more closely related to megabats than to the remaining 18 microbat families (Antrozoidae, Craseonycteridae, Emballonuridae, Furipteridae, Hipposideridae, Megadermatidae, Molossidae, Mormoopidae, Mystacinidae, Myzopodidae, Natalidae, Noctilionidae, Nycteridae, Phyllostomidae, Rhinolophidae, Rhinopomatidae, Thyropteridae, Vespertilionidae). This pattern of relationships has been confirmed by two comprehensive studies of phylogenetic relationships among mammals based on molecular evidence [Bininda-Emonds et al., 2007; Meredith et al., 2011]. A new cladistic classification reflecting these findings allocates Pteropodidae, Craseonycteridae, Hipposideridae, Megadermatidae, Rhinolophidae and Rhinopomatidae to the suborder Yinpterochiroptera and the remaining 18 microbat families to the suborder Yangochiroptera. To avoid confusion in what follows, the colloquial term "microbats" will be used for all bats excluding Pteropodidae.

Endocasts of Fossil Bats

As noted, very few specimens suitable for study of brain contours are available in collections. However, thanks to a short-term loan from the Basel Natural History Museum (BNHM), we were able to scan fossils allocated to 2 *Hipposideros* species. Members of the genus *Hipposideros* (Old World leaf-nosed bats) are now generally allocated to the family Hipposideridae, although previous classifications sometimes included them as a subfamily in Rhinolophidae. Hipposideridae and Rhinolophidae are

both families for which Niven [2005] specifically indicated that brain size reduction could be inferred from the analysis conducted by Safi et al. [2005].

Remains of hipposiderid bats, including a number of relatively complete skulls, are quite common in the Phosphorites de Quercy (Late Eocene-Middle Oligocene of France), actually accounting for the majority of fossil mammal specimens from those deposits. A prominent representative is *Hipposideros* (*Pseudorhinolophus*) *schlosseri*. Dechaseaux [1956] illustrated a natural endocast of this species but provided only linear dimensions; brain volume was not specified. Three relatively complete skulls of *Hipposideros schlosseri* were available for scanning in the present study (BNHM specimens QH118, QP787, QU269). The geological age of the deposits from which the specimens are derived is uncertain, but is probably close to the Eocene/Oligocene boundary (~35 Ma ago). A second species in the same genus and subgenus, *Hipposideros* (*Pseudorhinolophus*) *bouziguensis* is documented in the younger deposits of Bouzigues (France). In fact, this seems to be the last-surviving species of the subgenus *Pseudorhinolophus* [see Sigé et al., 1997]. The Bouzigues fauna is of Lower Miocene age and attributable to either the first interval (Aquitanian; 23.0-20.4 Ma ago) or the second (Burdigalian; 20.4-16.0 Ma ago). Curiously, many specimens of *Hipposideros bouziguensis* — the most abundant mammal species in the Bouzigues fauna — consist predominantly of natural endocasts. This facilitates determination of at least some brain contours even without scans, but makes inference of body mass problematic, as few cranial or dental structures are available for estimation. Edinger [1926] described two natural endocasts of *Hipposideros bouziguensis* and Dechaseaux [1938, 1956] portrayed a third. But for this species, too, only linear measurements were provided. In the present

study, three partially exposed natural endocasts of *Hipposideros bouziguensis* were subjected to scanning (BNHM specimens G2361a, G2361b, G2361d).

SRXTM investigations on the 6 fossil bat specimens were performed in March and December 2008, using the TOMCAT beamline [Stampanoni et al., 2006] of the Swiss Light Source at the Paul Scherrer Institut, Villigen, Switzerland. Scan settings differed between specimens and are listed in table 1. Tomographic reconstructions were computed using a highly optimized routine based on the Fourier Transform method [Marone et al., 2010]. The commercial software programmes Adobe Photoshop and VGStudio MAX were subsequently used to generate 3-dimensional images of bat endocasts from the tomographic scans.

To provide baseline information and guidance, scans of the skull of an extant bat (*Pipistrellus pipistrellus*) were acquired and used to generate initial reconstructions of cranial bone and the endocranial cavity (fig. 1). In addition to cranial bone, fossil scans generally included externally attached matrix and/or intrusive matrix that partially or completely filled the cranial cavity. So the programme Photoshop (Adobe Systems) was used to clean up each individual image by extracting just the outline of the brain cavity or natural endocast. VGStudio MAX was then used to load all scans together into 3D volumes and smooth the resulting figures. This programme was also used to calculate volumes of the 6 reconstructed bat endocasts.

Because slice-by-slice extraction of brain contours is a very laborious procedure, specification of reconstruction error is problematic. In order to check on measurement accuracy in the present study without time-consuming repetition of reconstructions, a virtual endocast for one fossil bat specimen was reconstructed independently by two

observers. The results obtained were 0.45 cm^3 and 0.44 cm^3, corresponding to a difference of just 2.3%.

Estimation of Body Mass in Fossils

Standard dental dimensions of skulls of 43 randomly selected extant bat species were measured with a small sliding caliper. Dental dimensions were exclusively derived from microbats. Wherever possible, average values were determined for 6 skulls for each species, although only 5 skulls were available in a few cases. Dimensions recorded included upper second molar length (M^2L) and width (M^2W), total length of the upper molars ($M^1L + M^2L + M^3L$) and the distance between right and left upper second molars. Molar dimensions have been widely used as predictors for body mass in a broad spectrum of mammals. Body mass values were taken from the PanTheria compilation of Jones et al. [2009]. In the fossils, linear measurements were taken directly from *H. schlosseri.* However, since only the natural endocasts were available for *H. bouziguensis*, it was necessary to use the average linear values cited by Sigé [1968] for remains of *H. bouziguensis* from Bouzigues.

Steps were taken to allow for the possible influence of phylogenetic inertia in quantitative comparisons. To determine an empirical formula for estimating body size in fossil bats, a phylogenetic tree was constructed by combining the mammal supertree of Bininda-Emonds et al. [2007] with a tree based on cytochrome *b* genes provided by Agnarsson [2011]. The better resolved Agnarsson tree was used as a starting-point, and 10 species were added from the Bininda-Emonds tree. Branch lengths were adjusted in order to yield an ultrametric tree, i.e. one in which all species exhibit the same distance

from the root of the tree. To estimate body size from dental dimensions, a phylogenetic generalized least squares (PGLS) approach using the package Comparative Analyses of Phylogenetics and Evolution in R (caper) [Orme et al., 2010; R Development Core Team, 2011] was applied. The best model was selected according to the Akaike information criterion (AIC).

Estimating Relative Brain Size

Having inferred body mass for the two fossil bat species, we calculated their relative brain sizes using extant bats as the point of reference. A tree for extant bat species was derived from Agnarsson et al. [2011], including only species for which both brain and body mass data are available (from Baron et al. [1996] and Jones and MacLarnon [2004], after excluding questionable data). Branch lengths were transformed to chronologic equivalents using the BEAST package (v1.6.2, Drummond & Rambaut, 2007). As in the *residuals first* method of Montgomery et al. [2010], residuals of brain versus body mass were calculated relative to a phylogenetically controlled empirical regression slope of 0.682.

Directionality of evolutionary trends

In order to investigate whether inclusion of the two fossil species influences the estimates of ancestral states, or recognition of a directional trend in the evolution of relative brain size, we used BayesTraits 1.1 [Pagel 1999, Pagel et al. 2004, including the former package CONTINUOUS]. We tested between a constant-variance random walk model and a directional random walk model, using both a Maximum Parsimony (MP)

and a Bayesian Markov Chain Monte Carlo (MCMC) approach. For MP, a likelihood test was performed to choose between these two models, and for MCMC, the harmonic means of the log-likelihoods of all models visited by the chain were compared by examining the BayesFactor. The testing procedures are described in detail by Montgomery et al. [2010], who conducted an analogous analysis in primates. However, to assess a directional trend in the evolution of a trait, the tree reflecting the phylogenetic relationships must show some variation in the root-to-tip lengths, i.e. the tree must not be ultrametric [Pagel 1997, 1999, Organ et al. 2007]. Therefore, such a test can only be performed in a tree that includes fossil species.

As shown in fig. 2, the two fossil species, *Hipposideros schlosseri* and *H. bouziguensis*, were inserted into the tree in a position basal to the node giving rise to all extant *Hipposideros* species together with *Asellia/Aselliscus/Coelops*. This position was constrained by the age of the fossils, which pre-dates the ancestral node for *Hipposideros Asellia/Aselliscus* species, but post-dates the time of divergence between that clade and *Triaenops*.

Because both fossil species examined are most probably related to extant Hipposideridae, Megadermatidae and Rhinolophidae, all tests were performed both for a reduced tree including only those 3 families (N=34 species) and for an overall tree for Chiroptera (N=199 species).

Reconstructing ancestral states

We applied two different methods to estimate the relative brain size of the most recent common ancestor of several clades: Maximum Parsimony in Mesquite (weighted

squared-change parsimony [Maddison and Maddison, 2011]) and the Bayesian Marcov Chain Monte Carlo (MCMC) approach in BayesTraits 1.1 [Pagel 1999, Pagel et al. 2004]. For the latter, the parameters of the model are estimated first, and in a second step the ancestral states for that model are calculated as the average of all posterior distribution values, excluding the first 500,000 values from the burn-in period. Ancestral states were estimated for trees both with fossils (N=199 species) and without fossils (N=197 species).

Results

Fossil endocast volume

Three-dimensional virtual endocasts were successfully reconstructed for all 6 fossil bat specimens scanned at TOMCAT (fig. 3). The following endocast volumes were determined: (1) *Hipposideros schlosseri* — specimen 1 = 0.24 cm^3, specimen 2 = 0.19 cm^3, specimen 3 = 0.27 cm^3. (2) *Hipposideros bouziguensis* — specimen 1 = 0.46 cm^3, specimen 2 = 0.44 cm^3, specimen 3 = 0.45 cm^3.

Fossil body mass estimates

Possibilities for estimation of body mass (BM) were limited because all fossil bat specimens studied were fragmentary. Upper molar length (M^2L) and width (M^2W) could be measured for all three specimens of *Hipposideros schlosseri*. But all three specimens of *Hipposideros bouziguensis* lacked teeth, so average values for M^2L and M^2W were taken from a monographic account of remains from Bouzigues by Sigé [1968]. Analysis using Phylogenetic Generalized Least Squares (PGLS) indicated that a combination of

M^2L and M^2W yielded better predictive power than either measurement alone (see Appendix for phylogenetic models and data). The following phylogenetically corrected allometric formula relating body mass (BM in g) to M^2L and M^2W (both in mm) was derived:

$$\log_e BM = 1.4897 * \log_e M^2L + 0.9096 * \log_e M^2W + 1.4853$$

This formula yielded an average body mass of 14.0 g for *Hipposideros schlosseri*. and an average of 28.7 g for *H. bouziguensis*.

Analysis of relative brain size

When included in a bivariate logarithmic plot of brain size against body mass for extant bats, values for the fossils (brain size from endocasts; body size estimated from upper second molar dimensions) fall into the lower region of the distribution (fig. 4). This is evident both relative to a least-squares regression fitted to the raw data and relative to a phylogenetically controlled line determined with PGLS. It is also noteworthy that relative brain size in the earlier, smaller-bodied fossil (Oligocene *Hipposideros schlosseri*) is somewhat lower than in the more recent, larger-bodied fossil (Miocene *H. bouziguensis*), as would be expected if relative brain size tends to increase over time.

Phylogenetic analysis with both Maximum Parsimony and Bayesian MCMC revealed that a directional model of evolution was more likely for relative brain size although this is not true either for brain size or for body size taken in isolation. In other words, a positive trend of relative brain size through evolutionary history of bats fits the data better than a random walk model (Table 2). The same result emerged regardless of whether analysis was conducted with the entire combined tree of Chiroptera (N=197

extant species) or with a reduced tree including only the 3 families Hipposideridae, Megadermrmatidae and Rhinolophidae (N=32 species). The result is highly significant with Maximum Parsiomony ($p < 0.0001$ and $p = 0.0001$, respectively). With Bayesian MCMC, a BayesFactor value >2 indicates a convincing result, so the high values obtained with all bats or with just the 3 families indicated (13.10 and 12.64, respectively) provide strong support for directional evolution of relative brain size.

Ancestral state estimations using Maximum Parsimony and Bayesian MCMC (Table 3) further indicate that the inclusion of fossils yields considerably lower ancestral values for nodes close in age to the fossil species (fig. 5). With deeper nodes, however, the fossils have less influence on parsimony estimates, and values approach those estimated without fossils. Bayesian MCMC, on the other hand, yields consistently low estimates of relative brain size for the deepest nodes when fossils are included. Overall, it seems likely that the relative brain size of the common ancestor of all bats was relatively small.

Discussion

The results of this first study of relative brain size in fossil bats provide no indication that any decrease has occurred in the genus *Hipposideros* over the past 35 Ma. This conflicts with the conclusion drawn by Safi et al. [2005] that evolutionary decrease in brain size has been widespread among bats, notably including members of the family Hipposideridae [Niven, 2005]. The findings for fossil *Hipposideros* are, instead, compatible with the interpretation that relative brain size has generally increased over time during mammalian evolution, albeit at different stages and at different rates. In fact,

phylogenetic analyses conducted with or without our data for relative brain size in two *Hipposideros* species provide statistically significant evidence for a general trend towards increased relative brain size during the evolution of bats.

In some mammalian lineages, notably in insectivores (both afrosoricidan and eulipotyphlan), expansion in brain size has been very limited. By contrast, in certain other lineages — especially in toothed cetaceans, canid carnivores and hominids — major expansions of relative brain size have taken place. A recent review by Shultz and Dunbar [2010], which explicitly included fossil evidence, confirmed the overall picture for mammals, ranging from very little change in some lineages to marked change in others. But their findings provided no indication that *relative* brain size has actually decreased in any lineage.

Relative brain size in certain extant insectivores characterized as "basal" (members of the families Erinaceidae and Soricidae) has often been taken as representative of the ancestral condition for placental mammals. However, while it is true that these insectivores generally have very small brains in comparison with other extant mammals, they are by no means unique in this. Many rodents, for example, have relative brain sizes lying within the "basal" insectivore range. Moreover, few attempts have been made to reconstruct the ancestral condition using actual fossil evidence. Ancestral placentals emerged at least 125 Ma ago and perhaps as far back as >160 Ma ago [Luo et al., 2011], and we need to infer relative brain size at the outset in order to trace the evolutionary history of encephalization among placental mammals with any confidence. The oldest fossil mammals included in the analysis conducted by Shultz and Dunbar [2010] date back to only ~60 Ma ago, leaving up to 100 Ma of prior evolutionary history

completely undocumented. Limited evidence for relative brain size in Cretaceous mammals (>65 Ma old) indicated that relative brain size was even smaller than in most or all extant insectivores [Martin and Isler, 2010], and this has now been confirmed by an analysis of improved evidence [Rowe et al., 2011]. Nonetheless, "basal" insectivores, various rodents and certain other mammals do provide a lower bound for relative brain size among mammals alive today.

As far as bats are concerned, it was established some time ago that frugivorous bats as a group have markedly larger brains, relative to body size, than insectivorous bats [Eisenberg and Wilson, 1978]. In fact, most values for relative brain size in frugivorous bats lie above the average for extant placental mammals, while the vast majority of values for insectivorous bats lie below that average level. Indeed, some microbats have relative brain sizes lying in the lower region of the distribution for extant placental mammals. So insectivorous microbats count among notably small-brained mammals. This can be explained in one of two ways. One possibility, raised by the claim made by Safi et al. [2005], is that extant insectivorous bats commonly have small brains because of widespread reduction from a larger-brained ancestry. However, the alternative possibility is that insectivorous bats have always been relatively small-brained and that comparatively little expansion occurred from the ancestral placental condition. If no convincing case can be made for reduction in brain size among bats, then the straightforward interpretation is that there was little scope for any such reduction from the ancestral chiropteran condition; their brains simply failed to expand to any marked degree. This is the interpretation supported by the findings presented here.

In contrast to our results, Safi et al. [2005], using the same procedures, found no evidence for a directional trend in relative brain size evolution in bats. However, at the time there were no fossil data available, and in the phylogenetic tree all branches between bifurcations had been assigned equal length. As a result, species in species-rich groups in their tree necessarily exhibit a longer pathway from root to tip than species in groups containing relatively few species. The directionality parameter in BayesTraits measures the regression of trait values across species against total path length from the root of the tree to the tips. Thus, Safi et al. [2005] actually tested whether species-rich groups differ in relative brain size from less speciose groups, and did not find any indication that this was the case.

It is in any case questionable whether change in a morphological character over time can be reliably inferred in the absence of fossil evidence. Analysis of body size in the mammalian order Carnivora by Finarelli and Flynn [2006] clearly illustrates potential pitfalls in reconstructing changes in size over time with exclusive reference to extant species. Among caniform carnivorans (Canidae, Ursidae, Pinnipedia, Musteloidea), many subgroups are now represented predominantly by large- or small-bodied species. The distribution of body sizes among extant species across the phylogeny seemingly indicates a pattern of decreasing body size from an ancestral value of 10-50 kg. However, body sizes estimated for fossil representatives of a given caniform taxon often lie well outside the observed ranges for extant members. So the present-day distribution of body sizes is not representative of the evolutionary history of the group. When 367 fossil taxa were included with 149 extant species in a combined analysis designed to reconstruct ancestral body sizes, a small-bodied ancestor (1-5 kg) was indicated both for Caniformia and for

the monophyletic subclade Arctoidea (Ursidae, Pinnipedia, Musteloidea). As Finarelli and Flynn [2006] noted: "Evolutionary trends can reduce the accuracy of character state reconstructions, especially for methods assuming Brownian motion as the model for character change. This is because an estimated root value under such a model will always be some form of weighted average of observed values for terminal taxa [Schluter et al., 1997], and if a trend moves the range of observed character state values beyond the ancestral condition, it will be difficult, if not impossible, to accurately reconstruct the condition at the ancestral node [Garland et al., 1999; Oakley and Cunningham, 2000]." At present, the most promising method is based on an adaptive peak model of evolution [Smaers and Vinicius 2009], which is, however, not yet available as a software package.

The topic of brain size reduction has attracted considerable interest since 2004 because of the discovery of fossil hominid remains attributed to the putative new species *Homo floresiensis* [Brown et al., 2004]. One remarkable feature of the primary Flores hominid specimen LB1 is its tiny brain size, which is quite unusual for a hominid that is only 18,000 years old. Various attempts have been made to explain the small brain of LB1 as a result of island dwarfism [Niven, 2007], or by referring to brain size reduction in other lineages [Dechmann and Safi 2009, Montgomery et al. 2010]. But several research groups have proposed instead that the LB1 individual was in fact a modern human suffering from a pathological condition [Weber et al., 2005; Martin et al., 2006a,b; Richards, 2006; Hershkovitz et al., 2007; Obendorf et al., 2008; Vannucci et al., 2011]. Our results demonstrate that including fossils in a reconstruction strongly supports a positive directionality in relative size of bat brains over time. Therefore, bats cannot be used as an argument to assign species status to LB1.

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Table 1: TOMCAT settings used for scanning individual specimens

specimen	**beam energy (keV)**	**expos. time (msec)**	**projections over 180^{o}**	**step angle (^{o})**	**magnific-ation**	**pixel size (μ)**
H. bouziguensis #1	40.005	150	1501	0.120	1	7.4
H. bouziguensis #2	40.005	150	1501	0.120	1	7.4
H. bouziguensis #3	40.007	2000	721	0.250	1.25	11.84
H. schlosseri #1	40.005	150	1501	0.120	1	7.4
H. schlosseri #2	40.005	150	1501	0.120	1	7.4
H. schlosseri #3	35.004	800	1001	0.180	1.25	11.84

Table 2: Results of tests for evolutionary trends in body mass, brain size and relative brain size. HMLL = Harmonic mean of log likelihood.

A. in all Chiroptera sampled (N=199 species)

	Maximum Parsimony			Bayesian MCMC		
Variable	Likelihood Ratio	p-value		HMLL random walk model	HMLL directional model	BayesFactor
Body mass	0.036	0.850		-219.88	-220.47	-1.18
Brain size	1.678	0.195		-152.55	-152.88	-0.66
Relative brain size	15.51	<0.0001		96.43	102.98	13.10

B. in the clade containing the fossils (N=34 species)

	Maximum Parsimony			Bayesian MCMC		
Variable	Likelihood Ratio	p-value		HMLL random walk model	HMLL directional model	BayesFactor
Body mass	0.028	0.867		-31.42	-32.44	-2.04
Brain size	1.492	0.222		-20.59	-20.79	-0.40
Relative brain size	14.454	0.0001		19.67	25.99	12.64

Table 3: Estimates of ancestral values of relative brain size for all bats, with fossils (N=199 species; before slash) or without (N=197 species; after slash)

	Parsimony	Bayesian MCMC
Node 181: *Hipposideros*	-0.128/-0.110	-0.210/-0.089
Node 147: *Rhinolophus*	-0.133/-0.113	-0.255/-0.067
Node 179: *Hipposideros* & *Asellia*/*Aselliscus/Coelops*	-0.402/-0.136	-0.301/-0.169
Node 178: Hipposideridae	-0.362/-0.076	-0.518/-0.088
Node 146: Rhinolophidae & Hipposideridae	-0.298/-0.059	-0.525/0.001
Node 142: Megadermatidae, Rhinolophidae and Hipposideridae	-0.193/-0.020	-0.599/-0.017
Node 141: Yinpterochiroptera	-0.127/-0.003	-0.533/-0.052
Node 3: Yinpterochiroptera & part of Yangochiroptera	-0.136/-0.053	-0.668/-0.097
Node 2: all Chiroptera	-0.170/-0.113	-0.847/-0.112

Figure 1. Reconstructed skull and 3 views of a virtual endocast of the extant bat *Pipistrellus pipistrellus* (common pipistrelle). Like many other microchiropteran bats, the common pipistrelle is very small, with a skull length of only 12.2 mm. High-resolution synchrotron-radiation X-ray tomographic miscroscopy is therefore needed for clear visualization. The endocast volume of this specimen is 110 mm^3, which agrees closely with an average brain mass of 95 ±10 mg reported for *P. pipistrellus* [Djavadian et al., 2006].

Figure 2. Inferred phylogenetic tree for extant Rhinolophidae and Hipposideridae, including the two fossil species *Hipposideros* (*Pseudorhinolophus*) *schlosseri* and *Hipposideros* (*Pseudorhinolophus*) *bouziguensis* with lineages indicated by heavy lines. (This is an extract from an overall phylogenetic tree for bats based on Bininda-Emonds et al. [2007] and Agnarsson et al. [2011].) Because the two fossil species are probably sister taxa and because their common ancestor would have existed ≥35 Ma ago, their lineage must have diverged at least 15 Ma prior to the node that gave rise to extant *Hipposideros, Asellia*, *Aselliscus* and *Coelops*, but after divergence of the lineage leading to *Triaenops* species.

Figure 3. Smoothed virtual endocasts reconstructed from 6 fossil bat specimens from 2 species of the family Hipposideridae: early Oligocene *Hipposideros* (*Pseudorhinolophus*) *schlosseri* (n = 3) and early Miocene *Hipposideros* (*Pseudorhinolophus*) *bouziguensis* (n = 3). In the specimens of *H. bouziguensis* natural endocasts were already exposed to varying degrees, so extraction only required removal of various skull fragments and attached matrix. Skulls of *H. schlosseri* were more

complete, so their endocasts had to be reconstructed from internal profiles of the braincase.

Figure 4. Bivariate logarithmic plot of brain mass (g) against body mass (g) for all available extant bat species (filled circles) and the two fossil species (open squares). Data for brain and body mass of extant bats are taken from Jones et al. [2009]. As explained in the text, body mass of the fossils was estimated from upper second molar dimensions. Best-fit lines shown are a least-squares regression fitted to the raw data (thin line) and a phylogenetically corrected line (PGLS; thick line). Both fossil species fall into the lower end of the distribution for extant bats.

Figure 5. Ancestral state estimations for relative brain size (logarithmic residual value) with fossils (filled squares) and without (open squares). Inclusion of the fossils consistently lowers the value for relative brain size inferred for ancestral nodes, both with Maximum Parsimony and with Marcov Chain Monte Carlo (MCMC).

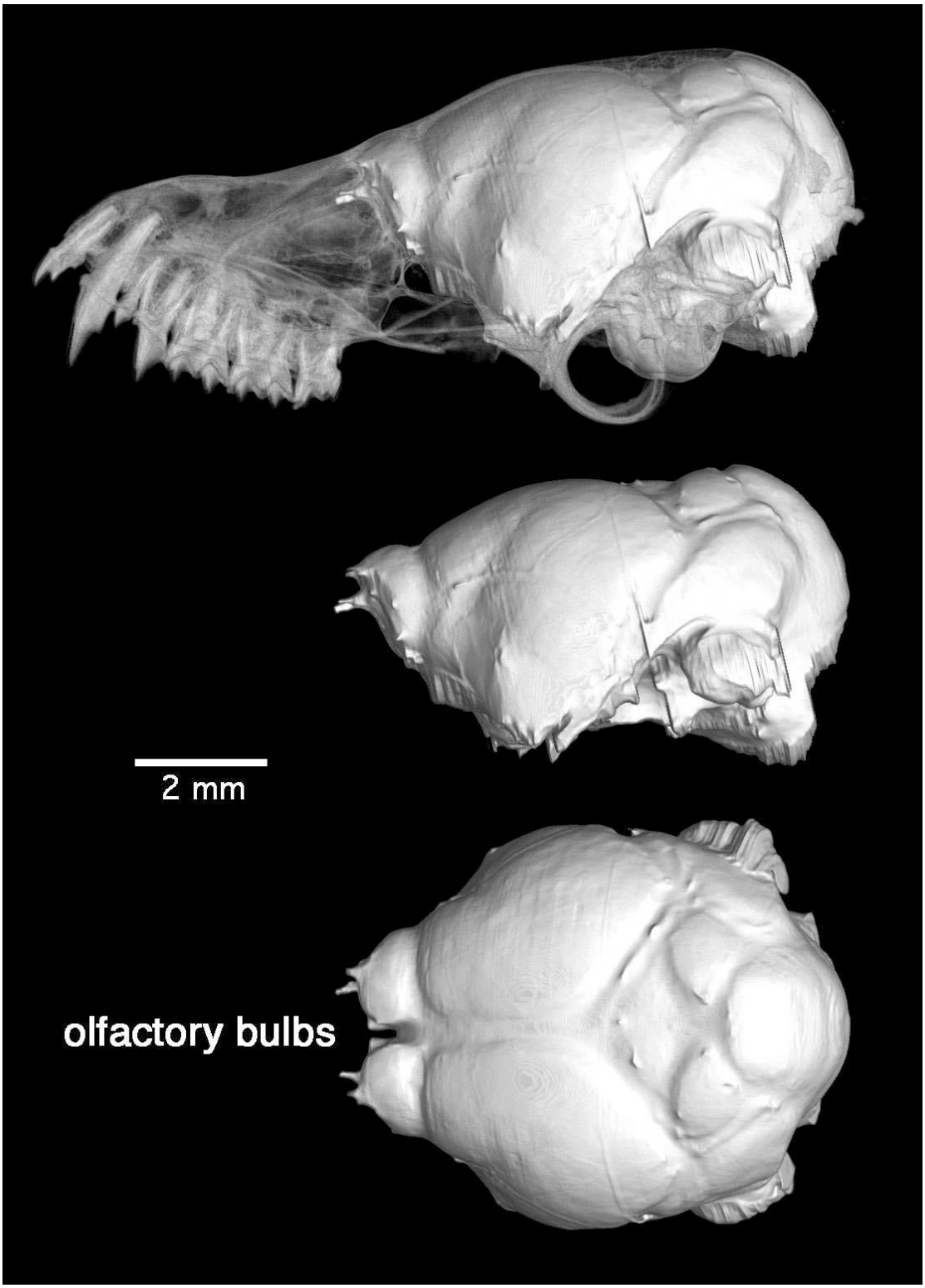


Figure 1

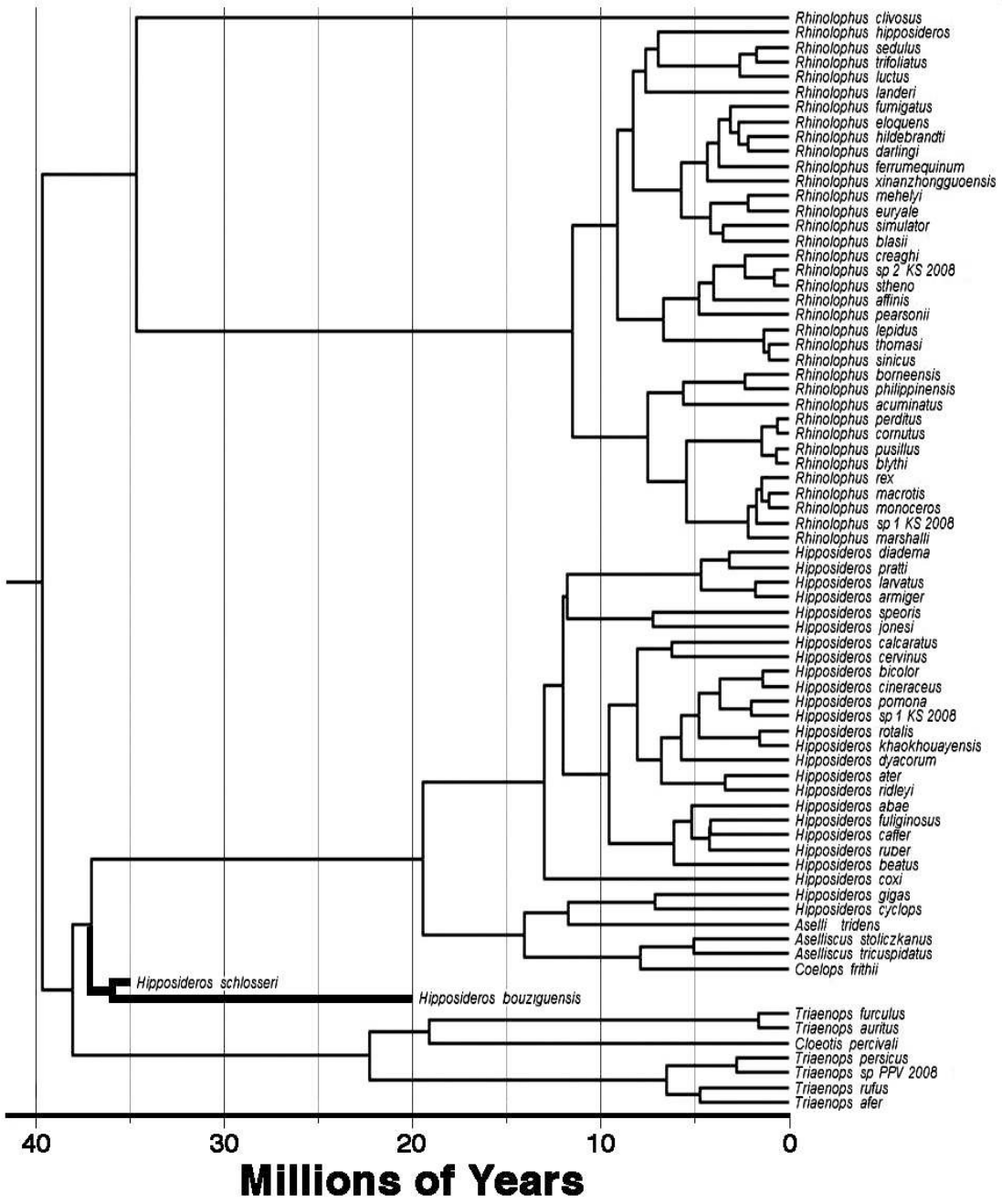
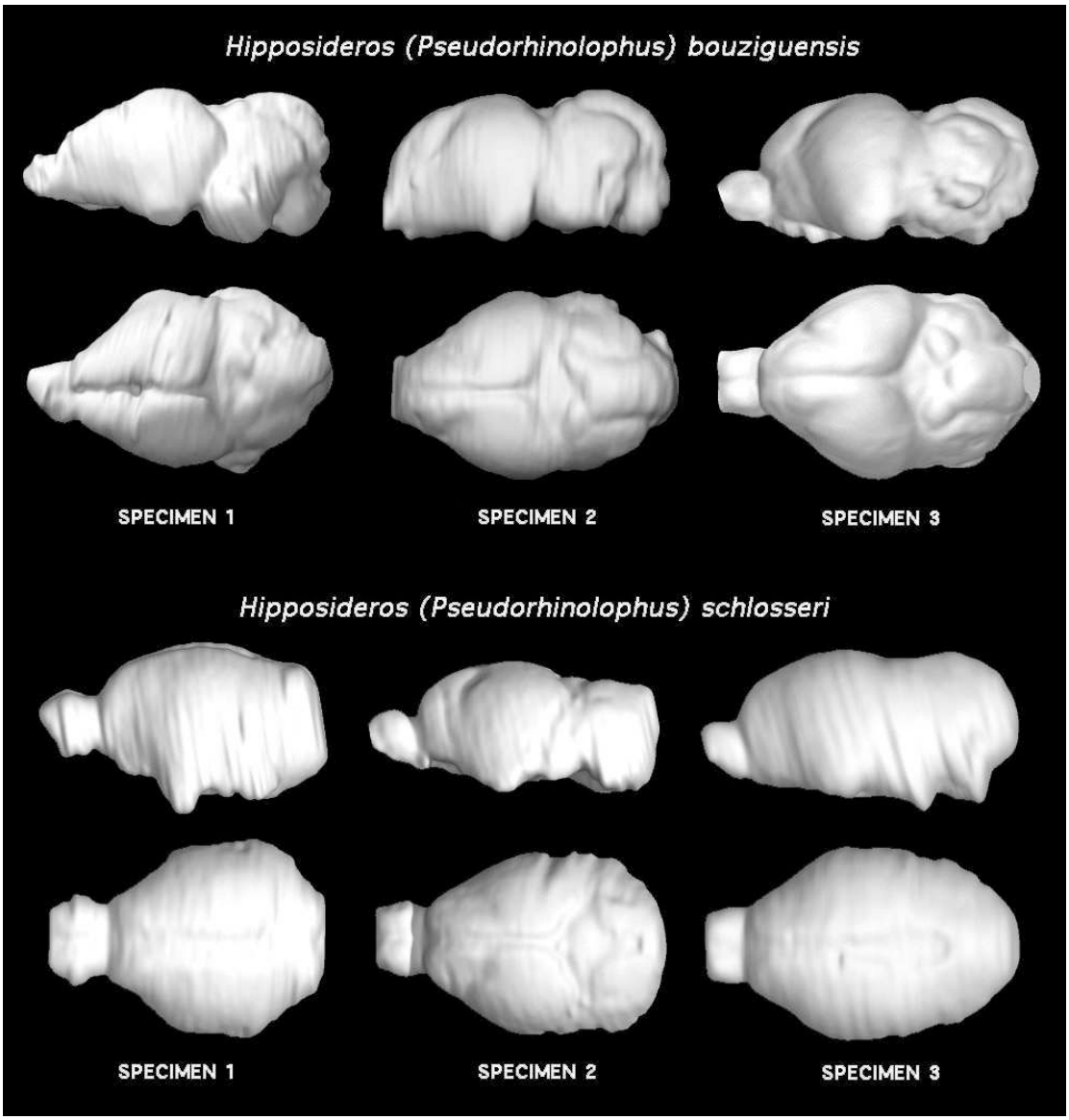

Rhinolophus clivosus
Rhinolophus hipposideros
Rhinolophus sedulus
Rhinolophus trifoliatus
Rhinolophus luctus
Rhinolophus landeri
Rhinolophus fumigatus
Rhinolophus eloquens
Rhinolophus hildebrandti
Rhinolophus darlingi
Rhinolophus ferrumequinum
Rhinolophus xinanzhongguoensis
Rhinolophus mehelyi
Rhinolophus euryale
Rhinolophus simulator
Rhinolophus blasii
Rhinolophus creaghi
Rhinolophus sp 2 KS 2008
Rhinolophus stheno
Rhinolophus affinis
Rhinolophus pearsonii
Rhinolophus lepidus
Rhinolophus thomasi
Rhinolophus sinicus
Rhinolophus borneensis
Rhinolophus philippinensis
Rhinolophus acuminatus
Rhinolophus perditus
Rhinolophus cornutus
Rhinolophus pusillus
Rhinolophus blythi
Rhinolophus rex
Rhinolophus macrotis
Rhinolophus monoceros
Rhinolophus sp 1 KS 2008
Rhinolophus marshalli
Hipposideros diadema
Hipposideros pratti
Hipposideros larvatus
Hipposideros armiger
Hipposideros speoris
Hipposideros jonesi
Hipposideros calcaratus
Hipposideros cervinus
Hipposideros bicolor
Hipposideros cineraceus
Hipposideros pomona
Hipposideros sp 1 KS 2008
Hipposideros rotalis
Hipposideros khaokhouayensis
Hipposideros dyacorum
Hipposideros ater
Hipposideros ridleyi
Hipposideros abae
Hipposideros fuliginosus
Hipposideros caffer
Hipposideros ruber
Hipposideros beatus
Hipposideros coxi
Hipposideros gigas
Hipposideros cyclops
Aselli tridens
Aselliscus stoliczkanus
Aselliscus tricuspidatus
Coelops frithii
Hipposideros schlosseri
Hipposideros bouziguensis
Triaenops furculus
Triaenops auritus
Cloeotis percivali
Triaenops persicus
Triaenops sp PPV 2008
Triaenops rufus
Triaenops afer
40
30
20
10
0
Millions of Years

Figure 2


Hipposideros (Pseudorhinolophus) bouziguensis
SPECIMEN 1
SPECIMEN 2
SPECIMEN 3
Hipposideros (Pseudorhinolophus) schlosseri
SPECIMEN 1
SPECIMEN 2
SPECIMEN 3
Figure 3

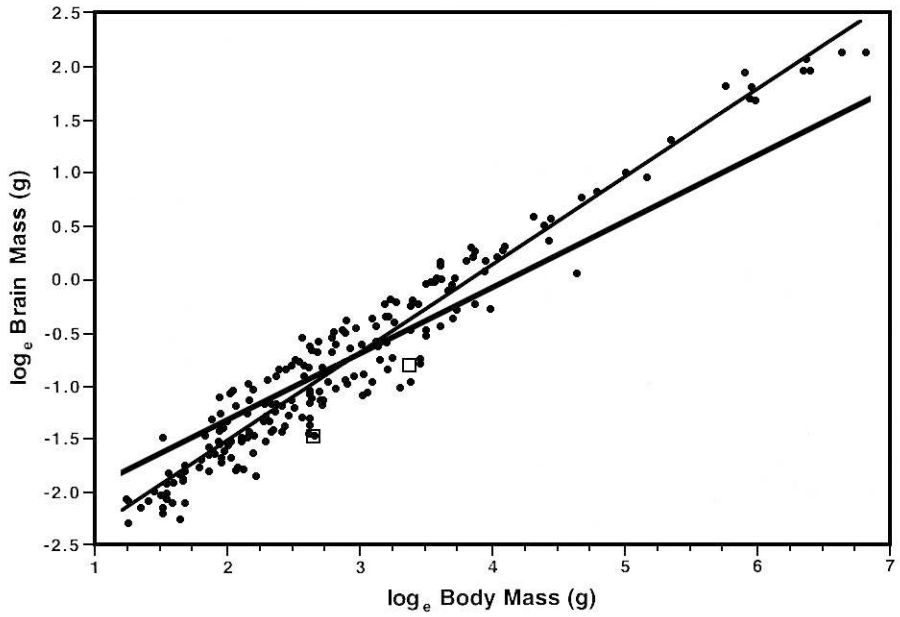

2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
log e Brain Mass (g)
1
2
3
4
5
6
7
log e Body Mass (g)

Figure 4

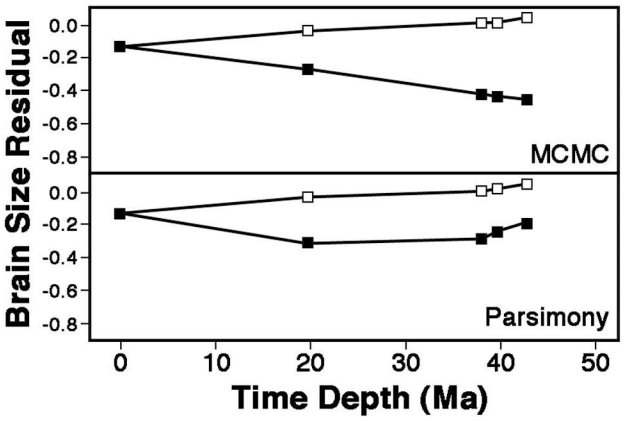

Brain Size Residual
0.0
-0.2
-0.4
-0.6
-0.8
MCMC
0.0
-0.2
-0.4
-0.6
-0.8
Parsimony
0
10
20
30
40
50
Time Depth (Ma)

Figure 5